

Recombinant protein purification and pulldown

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 An abbreviated version of this protocol was published in eLIFE in Oct 2014

The membrane-associated proteins FCHO and SGIP are allosteric activators of the AP2 clathrin adaptor complex

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Detailed protocol

2xYT culture media containing the appropriate antibiotics was inoculated with an overnight culture of bacterial cells expressing AP2 cores and incubated at 37°C with shaking at 180-200 RPM until OD₆₀₀ = ~1.0. The temperature was decreased to 18°C for 1 h before expression was induced with 100 µM isopropyl b-D-1-thiogalactopyranoside (IPTG) for 20-24 h. Cells were harvested by centrifugation, washed with lysis buffer (see below), and snap frozen in liquid N₂ prior to storage at -80°C. Cell pellets were resuspended in 50 mL lysis buffer per liter of initial culture volume. GST lysis buffer consists of PBS pH 7.4, 1 mM DTT, 60 µg/mL DNase I (grade II from bovine pancreas, Roche), 2.5 mM MgCl₂, 0.3 mg/mL lysozyme (Sigma), and 1 mM phenylmethylsulfonyl fluoride (PMSF) (Millipore, Billerica, MA) with the addition of 1 tablet of cOmplete EDTA-free Protease Inhibitor Cocktail (Roche) for every 100 mL. The cell slurry was sonicated (20 s pulses at 20% amplitude for a total of 8 min; 50 mL at a time) using a Q700 sonicator (Qsonica, Newtown, CT). Lysates were cleared by centrifugation (~20000 x g) and filtration (0.2 µm). Cleared filtrate was rotated for 1 h at 4°C with equilibrated GST resin (GE Healthcare Life Sciences, Uppsala, Sweden), 1 ml resin per liter of the initial culture volume. The filtrate and resin were poured over a gravity column, washed with PBS pH 7.4 + 1 mM DTT, and AP2 was eluted with 50 mM Tris, 150 mM NaCl, 10 mM reduced glutathione, and 1 mM DTT, pH 8.0 (Fischer BioReagents, Fairlawn, NJ). The eluate buffer was exchanged with TBS pH 7.6 + 1 mM DTT and AP2 cores were concentrated to ~0.5 mg/mL using an Amicon Centrifugal Filter Unit with a 100 kDa cutoff (Merck Millipore). Aliquots of AP2 cores were snap frozen in liquid N₂ prior to storage at -80°C.

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- Hollopeter, G. , Jorgensen, E. and Beacham, G. M.(2023). Recombinant protein purification and pulldown. Bio-protocol Preprint. bio-protocol.org/prep2159.
- Hollopeter, G., Lange, J. J., Zhang, Y., Vu, T. N., Gu, M., Ailion, M., Lambie, E. J., Slaughter, B. D., Unruh, J. R., Florens, L. and Jorgensen, E. M.(2014). The membrane-associated proteins FCHO and SGIP are allosteric activators of the AP2 clathrin adaptor complex. eLIFE. DOI: [10.7554/eLife.03648](https://doi.org/10.7554/eLife.03648)

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